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ON THE PRODUCTION OF PRECIPITINS.*

LUDVIG HEKTOEN.

(From the Memorial Institute for Infectious Diseases, Chicago.)

During the study of the production of antibodies under various conditions, some observations have been made on the development of specific precipitins that may be of interest.

Fornet and Müller[1] and others[2] have shown that when rabbits are injected intraperitoneally with increasing quantities of foreign serum on three successive days there results a fairly copious production of precipitins by about the twelfth day after the last injection. I have obtained the same results. Injected intraperitoneally on three successive days with the serum or blood of various animals (dog, horse, hog, beei, chicken, cat, rat, guinea-pig, fish, or man) in increasing quantities, usually 5, 10, and 15 c.c., one day apart, rabbits commonly produce specific precipitin enough by about the twelfth day after the third injection to cause precipitation in dilutions of the corresponding antigenic serum or blood of 1–1,000 and

* Received for publication February 2, 1914.

[1] *Ztschr. f. biol. Technik u. Methodik*, 1908, 1, p. 201.

[2] Bonhoff and Tsuzuki, *Ztschr. f. Immunitätsf., Orig.*, 1910, 4, p. 180; Gay and Fitzgerald, *Univ Cal. Publ. in Path.*, 1912, 2, p. 77.

upward to 1–20,000 and higher, and in the case of beef serum as high as 1–200,000 (Table 1).

TABLE 1.

SUMMARY OF RESULTS OF RAPID METHOD OF PRECIPITIN PRODUCTION.

Antigen	Quantity, Mode, and Place of Injection	Day of Test (After Last or Single Injection)	Titer
Human serum	5–10–15 c.c., one day apart. Intrap.	12	12,800
" "	" " " " " Intrav.	"	20,000
" "	" " " " " Intrap.	"	20,000
" "	" " " " " "	14	1,600
" "	" " " " " "	"	1,200
" "	" " " " " "	"	1,000
" "	" " " " " "	"	10,000
" blood	30 c.c. Intrap.	"	2,000
" "	" " "	"	16,000
" "	" " "	"	0*
Horse serum	15 " "	12	30,000
" "	5–10–15 c.c., one day apart. Intrap.	"	50,000
" " *	" " " " " Intrav.	"	20,000
" "	30 c.c. Intrap.	"	6,000
" "	" " "	11†	16,400
Sheep "	5–10–15 c.c., one day apart. Intrap.	13	10,000
" blood	" " " " " "	"	51,000
" "	" " " " " "	"	20,000
" "	" " " " " "	"	8,000
" "	30 c.c. Intrap.	14	16,000
Swine serum	5–10–15 c.c., one day apart. Intrap.	13	20,000
Beef "	5–10 " ‡ " " " "	12	200,000
" "	30 c.c. Intrap.	15	16,000
" "	" " "	"	12,800
Rat "	5–10–15 c.c., one day apart. Intrap.	"	2,400
" "	" " " " " "	10	20,000
Cat "	" " " " " "	14	10,000
Guinea-pig serum	" " " " " "	12	1,280
" "	" " " " " Intrav.	13	8,000
Fish "	" " " " " Intrap.	5§	2,560
Chicken "	" " " " " "	7	1,280
" "	" " " " " "	"	1,280
" blood	" " " " " "	13	60,000

* In this animal repeated tests were made during three weeks after the injection, but in no instance was any precipitate detected.

† The rabbit died on the eleventh day.

‡ Single injections of beef serum in quantities of 4–6 c.c. per kilogram of weight of rabbits gave antiserum of titer varying from 500–10,000 on the fourteenth to fifteenth day. The place of injection, whether intravenous or intraperitoneal, seemed to have no influence on this result.

§ This rabbit died on the sixth day.

The amount of antigen introduced by this method of triple injection was 30 c.c. in most cases. This amount of antigen in one injection, judging from a few experiments (Table 1), results in the production of about as much precipitin as when the antigen is given in three instalments one day apart.

The tests were made in small tubes with 0.5 c.c. of the diluted antigen and 0.1 c.c. of antiserum, care being taken to obtain a precise line of contact between the two fluids. The tubes were left at room temperature and the results recorded at the end of one hour. When tests were made with dilutions of whole blood the corpuscles

were first laked by means of water, and the normal salt content restored by the addition of the required amount of physiological salt solution of double strength, further dilutions being made with salt solution of usual strength. To secure perfect clearness filtration was used whenever necessary.

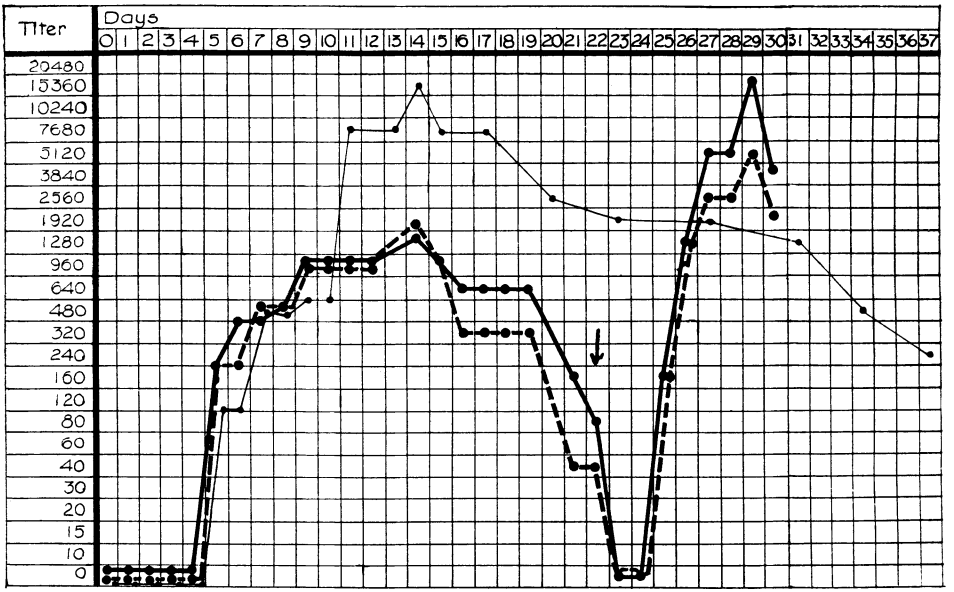

CHART 1.—Curves showing the course of specific precipitin in three rabbits injected with human serum or blood.

The rabbits represented by the heavy lines were injected peritoneally three times—on the first day with 5 c.c., on the second with 10 c.c., and on the third with 15 c.c. of human serum—and reinjected intravenously with 10 c.c. of human serum on the twenty-second day after the third injection. Day 1 is the first day after the third injection.

The figures under "titer" give the highest dilution of human serum in which the rabbit serum produced precipitate.

The rabbit represented by the fine line received 30 c.c. of defibrinated human blood on 0 day. In this case the figures under "titer" give the highest dilution human blood in which the rabbit serum produced precipitate.

It is obvious that under certain circumstances the rapid method of injecting antigen may have advantages of a practical nature over the older method of introducing the antigen at longer intervals. When old and young, healthy and more or less diseased, animals are used indiscriminately, both methods are subject to individual variations in the power to produce precipitins, but the rapid method

seemingly to no greater degree than the other. At all events, the fact that certain rabbits are found to produce comparatively little precipitin, no matter what method is used, makes it advisable always to immunize several at the same time, especially in case antihuman precipitin is to be produced as the response to human antigen occasionally may be comparatively slight.

The injection of the whole blood appears to produce just as much if not more precipitin than the injection of serum alone,

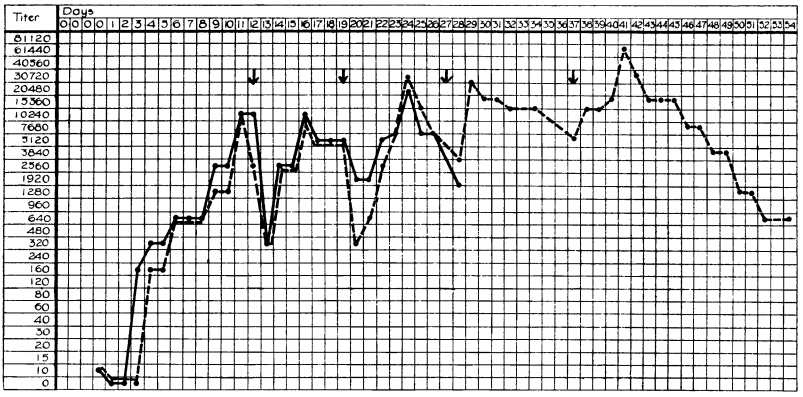

CHART 2.—Curves showing the course of specific precipitin in two rabbits injected with human serum and blood.

Three injections were made intraperitoneally of 5, 10, and 15 c.c. of human serum on three successive days respectively.

Day 1 on the chart is the first day after the last injection. On the twelfth day each received 20 c.c. of defibrinated human blood intraperitoneally; on the nineteenth, 30 c.c.; on the twenty-seventh, 40 c.c., and two days later one rabbit died; on the thirty-seventh day the remaining rabbit received 50 c.c. of human blood intraperitoneally.

The figures under "titer" represent the highest dilution of human blood in which precipitate was produced by the immune serum.

especially when tests are made with dilutions of whole blood. As we deal usually with whole blood rather than with serum only, in the identification of blood spots, it seems that it usually would be of advantage to use an antiserum produced by injection of the whole blood.

That the injection of carefully washed human corpuscles, which is made in order to obtain hemolytic amboceptor, also results in the production of precipitin for proteins in human serum (and blood) is shown by the following observations:

Case 49.—This rabbit received intraperitoneal injections of carefully washed human corpuscles as follows: April 1, the corpuscles of 3 c.c. of blood; April 6, of 5 c.c.; April 11, of 7 c.c.;

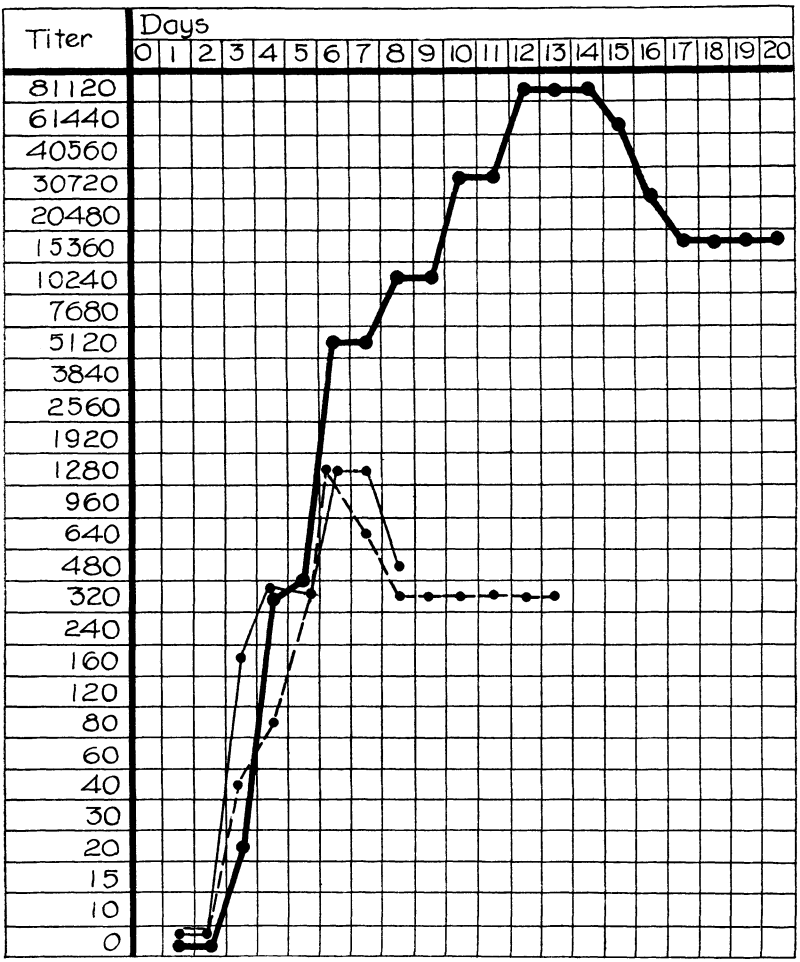

CHART 3.—Curves showing course of specific precipitin in rabbits injected with chicken blood or chicken serum

The fine lines refer to two rabbits, each of which was injected intraperitoneally with 5, 10, and 15 c.c. of chicken serum one day apart. The heavy line refers to a rabbit injected in the same way with the same quantities of defibrinated chicken blood.

Day 1 is the first day after the last injection.

The figures under "titer" give the highest dilution of chicken blood in which the immune serum produced precipitate.

April 16, of 9 c.c., and April 21, of 11 c.c. The titer of the serum on April 29 was 128,000 (human serum).

Case 61.—This rabbit received intraperitoneally the washed corpuscles of 5, 10, 15, and 20 c.c. of human blood in four injections

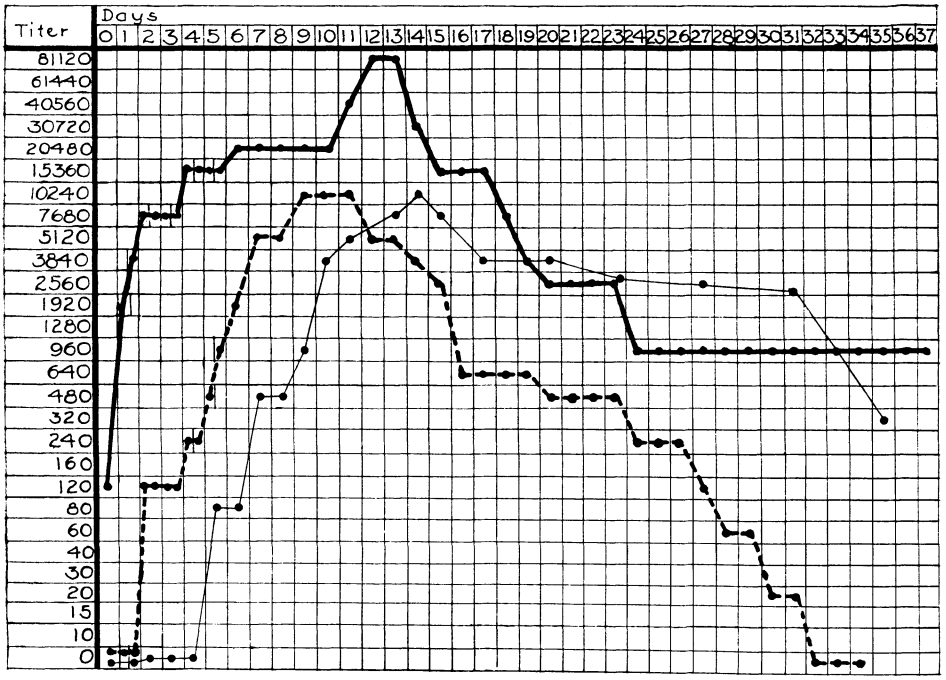

CHART 4.—Curves of precipitin and lysin and of precipitin in two rabbits injected with sheep blood.

Heavy solid line = lysin, heavy broken line = precipitin in rabbit injected intraperitoneally with 5, 10, and 15 c.c. sheep blood, one day apart. On the first five days two daily determinations were made about twelve hours apart. Day 1 is first day after last injection.

Fine line = precipitin in rabbit injected intraperitoneally at one time (day 0) with 30 c.c. of sheep blood.

The figures under "titer" give in the case of precipitin the highest dilution of sheep blood in which precipitate was produced by the rabbit serum; in the case of the lysin, the highest dilution in which the rabbit serum produced distinct lysis of sheep corpuscles.

In testing for the lysin, the rabbit serum was first heated at 58° C. for 30 minutes: the total quantity of each mixture was 0.6 c.c. of which 0.2 c.c. consisted of a 5 per cent suspension of washed sheep blood and 0.007 c.c. of fresh guinea-pig serum (complement), the rest being rabbit serum and salt solution.

four days apart. When tested with human serum ten days after the last injection the titer of the antiserum was 100,000.

When the course of the development of specific precipitin in the blood of rabbits immunized by the rapid method is followed by

daily tests under as strictly comparable conditions as possible, a curve is obtained that in all essentials corresponds to the typical curve described by the antibodies that develop after the single injection of other antigens under suitable conditions.

As shown by the charts, the acme is reached about the twelfth day or thereabouts after the last injection in the case of the triple

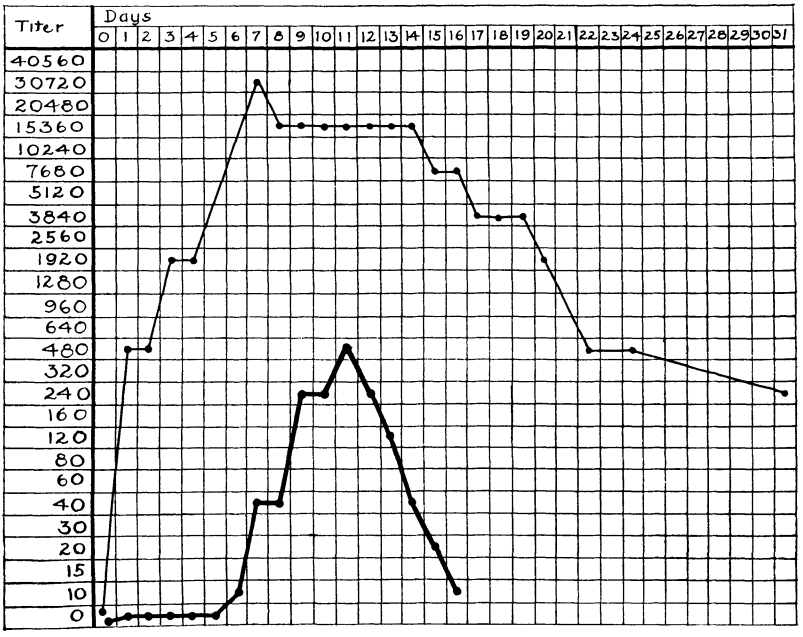

CHART 5.—Curves of precipitin (heavy line) and lysin (fine line) in dog injected with goat blood.

Three injections were made intraperitoneally—on the first day, 15 c.c., on the second, 30, and on the third, 45.

The figures under "titer" give in the case of precipitin the highest dilution of goat blood in which precipitate was produced by the dog serum; in the case of the lysin the highest dilution in which the dog serum produced distinct lysis of goat corpuscles.

In testing for the lysin, the dog serum was first heated at 58° C. for 30 minutes; the total quantity of each mixture was 0.6 c.c., of which 0.2 c.c. consisted of a 5 per cent suspension of washed goat blood and 0.0125 c.c. of fresh guinea-pig serum (complement), the rest being dog serum and salt solution.

method of injection, and as a rule a day or two later in the case of the single injection. The earliest apparent increase occurs rather abruptly, that is, in the course of a few hours, and most frequently on the third or fourth day, but sometimes a day or so earlier or later. There now comes a rapid rise at an increasing rate day by day until the acme is attained, whereupon there follows a gradual

decline. As shown in Chart 4, there may be a considerable rise in the course of 12 hours. When the curve remains low the period of latency may be prolonged.

Occasionally the curve without any apparent cause (infection?) begins to recede much earlier than is usually the case (see Chart 3).

As illustrated in Charts 1 and 2, subsequent injections of antigen may cause prompt decline in the amount of free precipitin in the blood, which is followed by an increase, the high point of which is often higher than the previous high point.

Chart 4 illustrates the fact that by means of the methods used the lysin is demonstrable earlier than the precipitin when the rabbits are injected with sheep blood, and also persists longer. Otherwise the courses of the two curves appear to be parallel.

The dog is known to be a poor producer of precipitin as compared with the rabbit, and this fact is illustrated in Chart 5, which gives the determination of the specific precipitin and lysin in a dog injected with goat blood.

SUMMARY.

By giving rabbits intraperitoneal injections of increasing quantities of serum or blood on three successive days a serviceable precipitating serum may be produced in about 15 days. The same quantity of antigen injected at one time also appears to give good results. The curve of the precipitin in such cases is like the simple antibody curve following a single injection of other antigens.

The injection of whole blood may be more advantageous in producing more precipitins for blood proteins in general than the injection of serum only.

The injection of washed human corpuscles gives rise to precipitins for human serum.